Remarks

Applicant notes with appreciation the Examiner drawing attention to the use of the term "process" in the use of Claims 11-23 and, as a result, has replaced the term "process" with the term "method." No new matter has been added.

Response to §112 Rejections

Claims 14, 15, 20, 21 and 23 have been rejected under 35 U.S.C. §112, second paragraph for being indefinite in failing to particularly point out and distinctly claim the subject matter. This rejection is respectfully traversed. The Applicant responds to such rejections in the order in which they were given:

A) Claims 14, 15 and 23 have been rejected as being indefinite to the extent of the scope encompassed by the term "protein derivative." Applicant respectfully submits that a term is to be given its plain meaning used by those of ordinary skill in the art. Applicant respectfully submits that a protein is recognized by those skilled in the art to mean:

> A large molecule composed of one or more chains of amino acids in a specific order; the order is determined by the base sequence of nucleotides in the gene coding for the protein. Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs.

Applicant further submits that "derivative" is understood to mean:

A substance that is made from another substance.

As such one skilled in the art would readily understand a "protein derivative" to be a large molecule composed of one or more chains of amino acids, which is made from another substance.

In view of this definition, the Applicant invites the Examiner's attention to page 6,

paragraphs 23 and 24, of the Applicant's specification where the term protein derivative is clearly defined. Specifically, a protein derivative is any derivative protein, which exhibits compacting property, as well as exhibiting properties associated with the histone proteins and other related proteins. Thus, one skilled in the art would readily understand that any protein derivative will share the structural and functional characteristics of the DNA compaction proteins, such as histone, envelope proteins, bacterial chromoid proteins, or any of a number of related proteins.

B) Claims 20 and 21 have been rejected as being indefinite for failing to define the amount of DNA compacting agent to be used, and for failing to clearly describe the formula listed in Claims 20 and 21. Applicant respectfully submits that the equation of Claims 20 and 21 clearly illustrate where the X and Y values are placed in their respective equations. Specifically, the X value is a power of 10, where the X has a range of 8-15. Y is a multiple having a range of 0.2-10.

As an illustrative example, the insertion of a value of 8 into the equation of Claim 20 results in a concentration of 10^{-8} (0.00000001) milligrams of compaction agent/ng total DNA/bp recombinant.

Applicant respectfully submits that the formula only discloses one variable in Claim 20 and two variables in Claim 21. Namely, the Applicant submits that the only variables in the equations of Claim 20 and 21 are for "X" and "Y." The equations described in Claims 20 and 21, as amended, refer to "ng total DNA" and "bp recombinant," which clearly relate to the units of measure for concentration of compaction agent.

The Examiner's attention is further invited to page 7, paragraph 35, where the specification illustrates that the concentration can be expressed in "mg of proteins per nanogram of total DNA contained in the ligation mixture and by base pair of recombinant." This unit of measure is

analogous to a concentration of solution, which can be expressed by, molarity (moles/liter); percent (weight/volume) – grams/100 ml; or percent via (volume/volume) – ml/100 ml. In these circumstances, the concentration of reagent in a solution can be obtained whether the amount of milligrams or milliliters of reagent or volume of solvent has changed. Claims 20 and 21 simply provide equations having a range of concentrations of compacting agent. As a result, "ng total DNA" and "bp recombinant" are not variables, but rather, units of measure.

Turning to the Examiner's assertion that the concentration formula does not impart clarity to the method claims, Applicant respectfully submits that the formula identifies the range of concentrations of compaction agent, which can be used to carry out the method step. Without this formula, one skilled in the art would be unaware of the proper range of concentration of compaction agents needed to complete the method steps.

C) Claim 13 has been rejected as failing to define "cellular medium." Applicant invites the Examiner's attention to page 4, paragraph 19, wherein the specification states that a cellular medium is any medium suitable for DNA cloning, such mediums being *E.coli* or yeast.

Finally, and in accordance with the Examiner's helpful suggestion, Applicant has amended Claim 13 to further define the cellular medium as "a medium suitable for cloning."

Response to §102 Rejections

Claims 11, and 14-23 have been rejected under 35 U.S.C. §102(b) as being anticipated by Sobczak et al. (Eur. J. Biochem. Vol. 175, pp. 379-385, 1988). Sobczak et al. analyzed the influence of a number of factors of intramolecular versus intermolecular ligation by using rate and extent of reaction as a measurement. Sobczak et al. specifically states:

"Many additional agents have been described for their ability to stimulate the rate and the extent of ligation into the influence of J/I ratio."

The solicited claims recite a method for preparing **circularized** recombinant nucleic acids in the presence of a compaction agent. Applicant respectfully submits that Sobczak et al. fails to show preparation of a **circularized** recombinant nucleic acid in the presence of a DNA compaction agent.

The first sentence of the Abstract of Sobczak et al. clearly illustrates that the study focused on the ligation of **linear** DNA. Sobczak et al. performed a study of the effect of histone H1 on ligation of cohesive and blunt DNA ends. Consequently, the linear products in Sobczak et al. are not cloning vectors and are not considered as such by their authors. Thus, the linear DNA taught in Sobczak et al. is not suitable for replication within E. coli.

Sobczak et al. observed the appearance "of high molecular mass concatemers," wherein it was well-known in the art that a concatemer is DNA molecule consisting of two or more separate molecules linked end-to-end to form a long linear structure.

The Examiner's attention is invited to page 6, paragraphs 27 and 28 of the Applicant's specification, which illustrate that histone H1 does not by itself, yield good results when acting as a compaction agent. Specifically, histone H1 is not involved in the forming of an octomer, which is helpful for the production of circularized recombinant nucleic acid via DNA compaction agents. Rather, histone H1 serves as a sealing histone to seal the DNA around the octomer. As a result, the compaction of circularized recombinant nucleic acid is not fully affected in the presence of histone H1, and consequently one skilled in the art following the protocol laid out in Sobczak et al. would not have expected production of circularized recombinant nucleic acid.

In further reference to the circularized products, Sobczak et al. does not describe recombinant vectors and/or cloning methods to prepare such circularized recombinants nucleic acid vectors. The current Office Action asserts that the linearization of pBR322 by HincII generates a vector and an insert. However, a study of Figs. 1D and 1E on page 381 of Sobczak et al. reveals that this specific linearization is only used in the furtherance of PEG to show that there is only concatemeriztion. As a result, Sobczak et al. states on page 380 that "In the case of blunt-end ligation of PBR322/HincII fragments,...intramolecular ligation did not occur (Fig. 1D and E)."

In the other experiments of Sobczak et al. and especially experiments having the presence of H1 histone, the linearized pBR322 vector was placed in a unique location by EcoRI (cohesive ends) or PvuII (blunt ends). (Sobczak et al. Figs. 4 and 5).

One important aspect of the present invention is to increase the number of recombinants in the presence of histones. Consequently, the Applicant showed results in the presence of histones as to compared to that in the absence of histones. The Applicant demonstrated that the increase in a number of recombinants was most likely a result from a stimulation at the vector's circularization step. In fact, the cloning of a 8159bp fragment and the result in increase of a number of recombinants in the presence of histones, has clearly demonstrated this effect. Consequently, without any test for cellular transformation and characterization of the recombinants, and without any recombination necessary, since there is no insert, the Applicants respectfully assert that the methods described in Sobczak et al. could not have led to the Applicants' results.

Sobczak et al.. have shown that the concatemerized products were increased in the presence of H1. However, Sobczak et al. show that other histones act upon ligation. In fact, the entire focus of these references is to illustrate H1 and its effect on DNA concatemerization. As a result, cloning

is not adapted to this study, because the linearized products are not vectors.

Lastly, the ligation data which is presented by Sobczak et al. is not quantified and only relates to a vector (pBR322), which is not meant to receive an insert. Thus, it would be impossible to assert that the cellular transformation would lead to an **increase** of the number of recombinants in the presence of histones. (Sobczak et al Fig. 4A, B, C and Fig. 5A, B).

As is described in Sobczak et al and supported fully by the Applicant's specification, circularized recombinants in the presence of H1 alone are unlikely, and any simulation effect under these conditions is also highly improbable.

In view of the foregoing, Applicants submit that the stimulatory effect of histones upon the number of recombinants, with the exception of H1, was not anticipated by Sobczak et al.

The Examiner's attention is further invited to page 379, second column of the materials of methods, wherein Sobczak et al. states "standard ligation was performed in fifteen micro liters, reaction mixtures containing... linear pbR322DNA substrate." In light of the foregoing, it is clear that Sobczak et al. fails to illustrate successfully preparing **circularized** recombinant nucleic acid in the presence of a DNA compaction agent.

The Applicant claims a method of preparing circularized recombinant nucleic acids capable of being ten killobases or greater through the use of a DNA compaction agent.

Accordingly, Applicant believes these rejections cannot be maintained and respectfully requests they be withdrawn.

Response to §103 Rejections

Applicant agrees that there is nothing novel about the fragmentation of a large kb segment.

It is well known in the art that one can cleave a sequence at two remote spots to give a large kb segment. However, Applicant asserts that the fragmentation of a large kb segment is not the inventive concept.

We note for the Examiner's convenience the decision of *In re Dow Chemical Co.*, 5 USPQ 2d, 1529,1531 (Fed. Cir. 1988) wherein the Federal Circuit Court, in setting out the appropriate test under 35 U.S.C. §103 stated that:

> "Where claimed subject matter has been rejected as obvious in view of prior art references, a proper analysis under §103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to one of ordinary skill in the art that they should make the claimed composition or device or carry out the claim process; and (2) whether the prior art would have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success."

In light of the remarks set forth in regard to the Sobczak et al. reference, Applicant respectfully submits that there is no motivation to combine the technique taught in Sobczak et al. with the vector disclosed by Gaffney et al. Specifically, nothing in Sobczak et al. hints to the potential of inserting the Sobczak linear concatemers into a vector. Furthermore, the Applicant's specification on page 3, paragraph 10, has specifically pointed out that there is no technique in the current art, which facilitated the circularization and preparation of vectors effective for moderate to large sizes of recombinant nucleic acid. Unlike the Applicant, Sobczak et al. does not teach the compaction properties of a variety histones or related proteins, other then H1. In light of the limited effect of histone H1 on compaction, the Sobczak et al. ligation technique would be unable to ligate large fragments into the vector described in Gaffney et al. Furthermore, Sobczak et al. does not contemplate nor suggest the insertion of a large linear concatemer into a circular vector. As a result,

Applicant respectfully submits that Claims 12 and 13 are clearly patentable over Sobczak et al. in view of Gaffney et al. Withdrawal of the 35 U.S.C. §103 is respectfully requested.

In light of the foregoing, Applicant respectfully submits that the entire application is now in condition for allowance, which action is respectfully requested.

Respectfully submitted,

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